February 25, 2003

Marine Biotoxins Program Manuscript Update

TOP STORIES:

**** "**NEW!!!**" denotes papers that are new to the manuscript update or have switched categories (ex. Submitted to In Press)

SUBMITTED:

** **NEW!!! MEASUREMENT OF BREVETOXIN LEVELS BY RADIOIMMUNOASSAY OF BLOOD COLLECTION CARDS AFTER ACUTE, LONG-TERM AND LOW DOSE EXPOSURE IN MICE.** (Environmental Health Perspectives) … Ricky Woofter, M-Yasmine Bottein Dechraoui, Ian Garthwaite, Neil R. Towers, Christopher J. Gordon , José Córdova and John S. Ramsdell

ABSTRACT

A radioimmunoassay (RIA) has been developed using a sheep anti-brevetoxin to evaluate detection of brevetoxin on blood collection cards from mice treated with the brevetoxin congener (PbTx-3). The RIA was designed in similar format to receptor assay to facilitate comparison with previous work with blood collection cards. The RIA uses a 1/4000 dilution of sheep antiserum, 0.4 nM [^{3}H]-PbTx-3, and goat antisheep IgG-cellulose with separation on glass fiber filters. The receptor binding assay (RBA), using rat brain membrane, has an affinity for PbTx-3 (EC_{50}= 4.3 ± 1.5 nM, n=7) and recognizes type 1 and type 2 brevetoxins, as well as ciguatoxin. Whereas the RIA, using a PbTx-2 specific antibody, has an affinity for PbTx-3 (EC_{50}= 1.2 ± 0.2 nM, n=10) and recognizes both type 1 and type 2 brevetoxins, but not ciguatoxin. Comparison of the different brevetoxin subtypes affinity using RIA and RBA yields a rank order of potency where PbTx 6 > 3 = 2 = 9 > 1. Thus, the two assays provide comparable values for the commonly occurring PbTx-2 and 3 as well as PbTx-9, while showing differences for PbTx-6 and PbTx-1. We next compared the two assays by measuring brevetoxin in the blood of mice exposed to a sublethal dose, 180 µg/kg of PbTx-3 for 0.5, 1, 2, 4, and 24 hr. The blood from each mouse was preserved on blood collection cards. Each 0.1 ml blood spot was extracted in 2 ml methanol. This extract was then tested by both assays. The RBA reported the blood brevetoxin activity (at 2 hr brevetoxin activity was detected in 3 of 4 mice), while the RIA gave blood brevetoxin levels (at 2 hr: 25.75, 28.27, 39.26, 28.51 nM PbTx-3). Taken together these results show the value of tier-based testing for brevetoxin: antibody methods provide a good screening method that may detect metabolites; receptor-based methods provide a good toxicity measurement and LC-MS/MS provides absolute confirmation of toxin congeners.

** NEW!!! **LEARNING IMPAIRMENT CAUSED BY A TOXIN PRODUCED BY *Pfiesteria piscicida* INFUSED INTO THE HIPPOCAMPUS OF RATS** (Neurotoxicology and Teratology)… Edward D. Levin et al. (Peter D. R. Moeller and John S. Ramsdell)

ABSTRACT

Pfiesteria piscicida, an estuarine dinoflagellate, which has been shown to kill fish, has also been associated with neurocognitive deficits in humans. With a rat model, we have demonstrated the cause-and-effect relationship between *Pfiesteria* exposure and learning impairment. In several studies, we have replicated the finding in Sprague-Dawley rats that exposure to fixed acute doses of *Pfiesteria* cells or filtrates caused radial-arm maze learning impairment. Recently, this finding of *Pfiesteria*-induced learning impairment in rats has been independently replicated in another laboratory as well. We have demonstrated significant *Pfiesteria*-induced learning impairment in both the win-shift and repeated acquisition tasks in the radial-arm maze and in reversal learning in a visual operant signal detection task. These learning impairments have been seen as long as 10 weeks after a single acute exposure to *Pfiesteria*. In the current study, we used a hydrophilic toxin isolated from clonal *Pfiesteria piscicida*

cultures (PfTx) and tested its effect when applied locally to the ventral hippocampus on repeated acquisition of rats in the radial-arm maze. Toxin exposure impaired choice accuracy in the radial-arm maze repeated acquisition procedure. The PfTx-induced impairment was seen at the beginning of the session and the early learning deficit was persistent across six weeks of testing after a single administration of the toxin. Eventually with enough practice each session the PfTx exposed rats did learn that session's problem as did control rats. This model has demonstrated the cause-and-effect relationship between exposure to a hydophillic toxin produced by *P. piscicida* and learning impairment and specifically that the ventral hippocampus was critically involved.

**** NEW!!! HARMFUL ALGAL BLOOMS: CAUSES, IMPACTS, AND DETECTION.**
(Journal of Industrial Microbiology) … K.G. Sellner, Greg Doucette and G. Kirkpatrick

ABSTRACT

Blooms of autotrophic algae and some heterotrophic protists are increasingly frequent in coastal waters around the world and are collectively grouped as harmful algal blooms (HABs). Blooms of these organisms are attributed to two primary factors: natural processes such as circulation,upwelling relaxation, and river flow; and, anthropogenic loadings leading to eutrophication. Unfortunately, the latter is commonly assumed as the primary cause for all blooms, which is not the case in many instances. Moreover, although it is generally acknowledged that occurrences of these phenomena are increasing throughout the world's oceans, the reasons for this apparent increase remain debated and include not only eutrophication but increased observation efforts in coastal zones of the world. There is a rapidly advancing monitoring effort resulting from the perception of increased impacts from these HABs, manifested as expanding routine coastal monitoring programs, rapid development and deployment of new detection methods for individual species, toxins, and toxicities, and expansion of coastal modeling activities towards observational forecasts of bloom landfall and eventually bloom prediction. Together, these many efforts will provide resource managers with the tools needed to develop effective strategies for the management and mitigation of HABs and their frequently devastating impacts on the coastal environment.

**** NEW!!! A RECEPTOR BINDING ASSAY FOR PARALYTIC SHELLFISH POISONING TOXINS: OPTIMIZATION AND INTERLABORATORY COMPARISON**
… Ruberu et al. (Greg Doucette and Christine Powell)

ABSTRACT

A receptor binding assay (RBA) for detection of paralytic shellfish poisoning toxins was formatted for use in a high throughput detection system employing microplate scintillation counting. The RBA technology was transferred from the National Ocean Service (NOS), which uses a Wallac TriLux 1450 MicroBeta microplate scintillation counter, to the California Department of Health Services (CDHS), which uses a Packard TopCount instrument. Due to differences in the detector arrangement between these two counters, markedly different counting efficiencies were exhibited, requiring optimization of the RBA protocol for the TopCount instrument. Precision, accuracy, and sensitivity (LOD = 0.2 mg STX equiv./100 g shellfish tissue) of the modified protocol were equivalent to those of the original protocol. The RBA robustness and adaptability were demonstrated by an interlaboratory study, in which STX concentrations in shellfish generated by the TopCount were consistent with MicroBeta-derived values. Comparison of saxitoxin reference standards obtained from the FDA and the National Research Council, Canada showed no observable differences. This study confirms the RBA's value as a rapid, high throughput screen prior to testing by the conventional mouse bioassay (MBA) and suitable for providing an early warning of increasing PSP toxicity when toxin levels are below the MBA limit of detection.

**** TYPE B BREVETOXINS SHOW TISSUE SELECTIVITY FOR VOLTAGE-GATED SODIUM CHANNELS: COMPARISON OF BRAIN, SKELETAL MUSCLE AND CARDIAC SODIUM CHANNELS.** (Toxicon)
… Marie-Yasmine Bottein Dechraoui and John S. Ramsdell

ABSTRACT

Brevetoxins and ciguatoxins are two classes of phycotoxins which exert their toxic effect by binding to site-5 of voltage-gated sodium channels. Sodium channels, a family of at least ten structurally different proteins, are responsible for the rising phase of the action potential in membranes of neural, cardiac and muscular excitable cells. This work is a comparative study of the binding properties and the cytotoxic effect of ciguatoxins and brevetoxins on human embryonic cells (HEK) stably expressing either the skeletal muscle ($Na_v1.4$), or the cardiac ($Na_v1.5$) sodium channel α-subunit isoforms. We report that type A (PbTx-1) and type B (PbTx-3 and PbTx-2) brevetoxins as well as ciguatoxins target both cardiac and muscle channels; type B brevetoxins show isoform selectivity, presenting a lower affinity for the heart than the skeletal muscle channel. The lower selectivity of type-B brevetoxins for heart sodium channels may result from a more rigid backbone structure than is found in type-2 brevetoxins and ciguatoxins.

**** PHYLOGENETICS AND rRNA PROBE DESIGN FOR ALGICIDAL BACTERIA ACTIVE AGAINST *Karenia brevis* (Dinophyceae)** (In process of re-submission)… Xavier Mayali and Greg Doucette

ABSTRACT

Two strains of bacteria isolated from the Gulf of Mexico and determined to be algicidal against the harmful algal bloom (HAB) forming dinoflagellate *Karenia brevis* were phylogenetically classified using 16S rDNA data. A novel statistical analysis of these strains as well as additional algicidal bacteria associated with blooms of other HAB species revealed 3 phylogenetically distinct clades abundant in such bacteria, within the genera *Cytophaga*, *Alteromonas*, and *Pseudoalteromonas*. This pattern is consistent with the hypothesis that independent radiation events took place during the course of algicidal bacteria evolution, suggesting that algicidal activity could have been an adaptive trait, and supporting the idea that algicidal bacteria may play a role in algal bloom dynamics. A strain-specific rRNA probe was designed for one algicidal bacterium (strain 41-DBG2) and using fluorescent *in situ* hybridization (FISH), was successful in identifying this strain in laboratory and field samples enriched for algicidal bacteria. In addition, one sample from a natural *K. brevis* bloom exhibited a positive signal using this method. Denaturing gradient gel electrophoresis (DGGE) was applied as an additional culture-independent method capable of identifying algicidal bacterium 41-DBG2 in mixed bacterial assemblages. DGGE also revealed more complex microbial communities in *K. brevis* bloom samples compared to 6 laboratory clonal isolates. Several of the latter also appeared to share phylotypes with one another, suggesting that similar bacteria may be associated with *K. brevis* cultures originating from different locations.

IN PRESS:

**** NEW!!! EXPRESSION OF A α,β,γ TUBULIN, THE MINIMAL SET OF TUBULINS REQUIRED TO DEFINE MICROTUBULE FUNCTION IN EUKARYOTIC CELLS, IN THE UNICELLULAR DINOFLAGELLATE, *Karenia brevis*.** (Phycologia) … Michèle Barbier et al. (Jeanine Miller, Steve Morton and Fran VanDolah)

ABSTRACT

Tubulin is a highly conserved family of proteins that are a major component of the microtubule cytoskeleton of eukaryotic cells. Here we report the presence of the three essential members of this family, α-, β- and γ-tubulin, in the unicellular dinoflagellate *Karenia brevis* by western blotting and immunolocalization. The cortical cytoskeleton and the intracytoplasmic structures are detailed by immunocytofluorescence techniques using antibodies to each tubulin on whole-permeabilized cells from laboratory cultures or field samples. The cortical microtubules could be visualized with anti-α- and anti-β-tubulin labeling revealing a morphology typical of dinoflagellates, while γ-tubulin was detected near the nucleus, probably associated with the archoplasmic sphere. The mitotic spindle, which arises from this region is described during different stages of mitosis. The cortical cytoskeleton does not depolymerize during mitosis, a feature that appears to be unique to dinoflagellates. For the first time, a detailed description of the cytoskeleton and the mitotic process is presented in the dinoflagellate *K. brevis*.

** NEW!!! EXPRESSION OF A CYCLIN B HOMOLOGUE IN THE CELL CYCLE OF A PRIMITIVE DINOFLAGELLATE, *K. brevis* …(Journal of Eukaryotic Microbiology)

Michèle Barbier et al. (Tod Leighfield and Fran VanDolah)

ABSTRACT

The eukaryotic cell cycle is driven by a set of cyclin dependent kinases associated with their regulatory partners the cyclins, which confer activity, substrate specificity and proper localization of the kinase activity. We describe the cell cycle of *Karenia brevis* and provide evidence for the presence of a cyclin B homologue in this primitive eukaryotic dinoflagellate. This cyclin B homologue has an unusual behavior, since its expression is permanent and its localization is cytoplasmic throughout the cell cycle. This behavior is similar to a cyclin B homologue, p56, previously described in a different species of dinoflagellate. However, in *K. brevis*, the cyclin B homologue is also present in the nucleus, specifically bound to the nucleolus during interphase. There is no evidence for the translocation to the nucleus during mitosis. Here we discuss the unique behavior of the cyclin B homologue in dinoflagellates, its relationship to the unusual characteristics of dinomitosis, and its potential implications regarding the evolution of cell cycle regulation among eukaryotes.

** CULTURE METHODS (Chapter in Manual of Harmful Marine Microalgae)…R. R. L. Guillard and S.L. Morton

ABSTRACT

Not available in electronic form at this time. See Steve Morton.

** USE OF CELL-SPECIFIC PAM-FLUOROMETRY TO CHARACTERIZE HOST SHADING IN THE EPIPHYTIC DINOFLAGELLATE *Gambierdiscus toxicus* (Journal of Marine Ecology) …Tracy A. Villareal and Steve L. Morton

ABSTRACT

Cell-specific fluorescence characteristics were used to characterize the light tolerance of the toxic benthic dinoflagellate *Gambierdiscus toxicus*. The fluorescence parameter F_v:F_m was measured on individual cells collected from foliose red algae growing in the sub-tidal margin of Southwater Cay, Belize. Samples were collected over several days during sunny and cloudy conditions and compared to samples *in-situ*. The data from individual cells was used to generate both frequency histograms and averages. Maximum individual cell (F_v:F_m) values reached 0.81 in pre-dawn samples, a value near the theoretical maximum for PAM fluorometry. In field samples, average F_v:F_m declined only slightly during the day, but cells incubated under 47% sunlight showed a significant mid-day depression. In freshly collected samples, near-maximum F_v:F_m could be found in individual cells at all time points; however, the frequency histograms indicated a great range in F_v:F_m at all time points. In contrast, cultures showed a tight distribution around a mean. Field samples showed a rapid recovery to near maximum F_v:F_m when assayed using a standardized actinic light series. Similar results were found in laboratory culture grown at 73 µmol m^{-2} s^{-1},

but not at 383 µmol m^{-2} s^{-1}. These data provide empirical support for suggestions that *G. toxicus* exploits the 3-dimensional structure of the algal host thallus to minimize light. This strategy permits *G. toxicus*, a high-light intolerant species, to thrive in shallow, well-lit tropical seas.

PUBLISHED:

** NEW!!! MICROBIAL COMMUNITY INTERACTIONS AND POPULATION DYNAMICS OF AN ALGICIDAL BACTERIUM ACTIVE AGAINST *Karenia brevis* (Dinophyceae) (Harmful Algae. 1(3):277-293)… Xavier Mayali and Greg Doucette

ABSTRACT

We investigated the population dynamics of *Cytophaga* strain 41-DBG2, a bacterium algicidal to the harmful algal bloom (HAB) dinoflagellate *Karenia brevis*, in laboratory experiments using fluorescent *in situ* hybridization (FISH) and denaturing gradient gel electrophoresis (DGGE). Following its introduction into non-axenic *K. brevis* cultures at concentrations of 10^3 or 10^5 cells ml^{-1}, 41-DBG2 increased to 10^6 cells ml^{-1} before the initiation of its algicidal activity. Such threshold concentrations were not achieved when algal cell numbers were low, suggesting that the growth of this bacterium required high levels of dissolved organic matter (DOM) excreted by the algae. It remains to be determined whether the threshold concentration is required to trigger an algicidal response by 41-DBG2 or alternatively, is the point at which the bacterium accumulates to an effective killing concentration. The microbial community, as determined by DGGE profiles, did not change until after *K. brevis* cells were in the process of lysing, indicating a response to the rapid input of algal-derived organic matter. We found that the resistance to algicidal attack exhibited by several *K. brevis* clones was due to the inhibition of 41-DBG2 growth in the presence of currently uncultivable bacteria associated with those clones, consequently preventing 41-DBG2 from reaching its threshold concentration required for algicidal activity. Remarkably, immunity and susceptibility to the algicidal activity of 41-DBG2 could be exchanged between *K. brevis* clones with the transfer of their respective unattached bacterial communities, and several dominant phylotypes sequenced from these communities belonged to the α-Proteobacteria, γ-Proteobacteria, and Cytophaga-Flavobacterium-Bacteroides (CFB) groups. We hypothesize that the CFB bacteria may be successfully competing with 41-DBG2 (also a member of the CFB) for nutrients, thereby inhibiting the growth of the latter and indirectly providing immunity against algicidal attack. We conclude that if algicidal bacteria play a significant role in HAB dynamics, as some authors have inferred, bacterial community interactions are crucial factors that must be taken into consideration in future studies.

** NEW!!! EVIDENCE FOR A CAMP-DEPENDENT PROTEIN KINASE IN A DINOFLAGELLATE, *Amphidinium operculatum* (Comp. Biochem. Physiol. B. 133:2317-2324) … Tod Leighfield, Michèle Barbier and Fran M. Van Dolah)

ABSTRACT

A cAMP dependent protein kinase (PKA) was identified in the dinoflagellate *Amphidinium operculum*. *In vitro* kinase activity towards kemptide, a PKA-specific substrate, was not detectable in crude lysates. However, fractionation of dinoflagellate extracts by gel filtration chromatography showed PKA-like activity toward kemptide

at approximately 66 kDa. These findings suggest that possible low molecular weight inhibitors in crude lysates were removed by the gel filtration chromatography. Pre-incubation of extracts with cAMP prior to chromatography resulted in an apparent molecular weight shift in the *in vitro* kinase assay to 40 kDa. An in-gel kinase assay reflected activity of the free catalytic subunit at approximately 40kDa. Furthermore, Western blotting with an antibody to the human PKA catalytic subunit confirmed a catalytic subunit with a mass of approximately 40kDa. Results from this study indicate that the PKA in *A. operculatum* has a catalytic subunit of similar size to that in higher eukaryotes, but with a holoenzyme of a size suggesting a dimeric, rather than tetrameric structure.

** NEW!!! **SODIUM CHANNEL ISOFORM-SPECIFIC TOXINS, IMPLICATION FOR TOXICOLOGICAL ANALYSIS** (Toxines et Recherches Biomedicales, Elsevier, Paris. pp. 35-44)… Marie-Yasmine Bottein Dechraoui and John S. Ramsdell

ABSTRACT

Arrays of biological compounds exert their toxic effect by binding to specific sites on voltage-gated sodium channels, providing potent chemical agents for defense or to kill their prey, but also inducing potent human intoxication or envenoming. Probably present in cells of all life forms, sodium channels are expressed to a greater extent in nerve, heart and skeletal muscle cells where they play a central role in the generation and propagation of action potentials. In mammals, a family of ten sodium channel α-subunits has been documented through isolation of separate cDNA and subsequent amino acid assignment. Sodium channels can be differentiated by their primary structure, their kinetics as well as their pharmacologic properties such as their relative sensitivity to a specific neurotoxin. They were initially distinguished as two subtypes according to their sensitivity to tetrodotoxin. However, through the expression of functional sodium channel subtypes in cell systems, other toxins also reveal further selectivity. This review of toxin interaction with voltage gated sodium channels, examines toxin binding sites, toxin selectivity for channel isoforms and implications of emerging research for toxiciological analysis

** NEW!!! **PARALYTIC SHELLFISH POISONING IN THE ABALONE *Haliotis midae* ON THE WEST COAST OF SOUTH AFRICA[5]** (Journal of Shellfish Research. 20:895-904)…Grant C. Pitcher, J.M. Franco, Greg Doucette, Christine Powell and A. Mouton.

ABSTRACT

In April 1999, monitoring on two abalone farms on the West Coast of South Africa provided evidence of the presence of PSP toxins in the cultured abalone *Haliotis midae*. Subsequent analysis of wild animals collected from the West Coast also revealed the accumulation of PSP toxins in these gastropods. The toxicity of individual animals as measured by the AOAC mouse bioassay showed considerable variation, ranging from below the assay detection limit to a maximum of 1609 µg STX eq 100 g^{-1}. Initial observations found PSP toxins in abalone to be coincident with blooms of *Alexandrium catenella* indicating that this dinoflagellate was the probable cause of abalone toxicity. Subsequent detection by receptor binding assay, of toxicity in abalone on the South Coast, an area considered free of *A. catenella* blooms, casts some doubt as to the source of the toxins. The toxin composition in the abalone as determined by HPLC was dominated by STX, and differed significantly from the toxin profile of *A. catenella* and the co-occurring mussel, *Mytilus galloprovincialis*. These findings indicated either a high capacity for biotransformation of PSP toxins by abalone or that *A. catenella* was not the source of the toxin. Investigation of the anatomical distribution of toxins revealed that they were not evenly distributed throughout the abalone tissues, but appeared to concentrate in outer layer tissue. The muscular foot made a disproportionately low contribution to the total toxin content of the mollusc, whereas the epipodial fringe, although comprising a small proportion of the abalone total weight, contributed substantially to the total toxin content. The epipodial fringe is typically included with the muscular foot as that part of the animal marketed for human consumption. The negative impacts of PSP contamination on abalone spawning and larval survival are presented and the findings of this study are compared to observations of PSP toxins in the abalone *Haliotis tuberculata* on the Galician coast. The inability of abalone to detoxify or depurate accumulated PSP toxins below the regulatory level threatens the future of the established abalone fishery and the newly developed aquaculture operations on the West Coast of South Africa.

** NEW!!! **KRILL: A POTENTIAL VECTOR FOR DOMOIC ACID IN MARINE FOOD WEBS** (Marine Ecology Progress Series. 237:209-216)…Sibel Bargu, Christine Powell, S.L. Coale, Mark Busman, Greg Doucette and M.J. Silver.

ABSTRACT

Over the past decade, blooms of the domoic acid (DA)-producing diatom *Pseudo-nitzschia* have been responsible for numerous deaths of marine mammals and birds in Monterey Bay, CA. Pacific euphausiids (krill) are important members of the local zooplankton grazer community and comprise the primary diet of squid, baleen whales, and many seabirds. Krill are thus a key potential vector for the transfer of DA to higher trophic level organisms in Monterey Bay. A better understanding of the quantitative trophic interactions and body burden of DA in krill is required to predict whether they can act as an effective vector for this neurotoxin. Here we report results of toxin analyses and gut content examinations of krill, collected from Monterey Bay, CA, in 2000. Corresponding counts of toxic *Pseudo-nitzschia* species in the water and their cellular DA concentrations were also obtained at the collection sites. Toxin analysis by receptor binding assay demonstrated that DA in krill tissue varied between 0.1 – 44 µg DA equiv g^{-1} tissue (confirmed by tandem mass spectrometry) depending upon the abundance of toxic *Pseudo-nitzschia* species in the water. The occurrence of *Pseudo-nitzschia* frustules in the digestive tract of krill verified that a toxic species of this diatom was an important part of their diet and thus implicated this phytoplankter as the source of domoic acid. These findings provide, for the first time, compelling evidence for the role of krill as a potential transfer agent of the phycotoxin DA to higher trophic levels in marine food webs.

** NEW!!! **DEVELOPMENT OF A PROTOCOL FOR DETERMINATION OF DOMOIC ACID IN THE SAND CRAB (*Emerita analoga*): A POSSIBLE NEW INDICATOR SPECIES**[4] (Toxicon 40:481-488)…Christine Powell, M.E. Ferdin, Mark Busman, R.G. Kvitek, and Greg Doucette.

ABSTRACT

The aim of this study was to begin evaluating the utility of mole crabs (*Emerita analoga*) as an indicator species for the algal neurotoxin, domoic acid (DA), in Monterey Bay, California, USA, a site of recurrent blooms of the DA-producing diatom *Pseudo-nitzschia*. One of the current sentinel organisms, the intertidal blue mussel (*Mytilus edulis*), shows minimal or undetectable toxicity during some local bloom events. As a critical step in assuring the accuracy of DA determinations in *E. analoga*, we have developed and validated a highly efficient extraction protocol that yields toxin recoveries of 97 $\pm$ 2.9 percent. We also determined by HPLC-UV and receptor binding assay, with confirmation by LC-MS/MS analyses, that mole crabs accumulated measurable amounts of DA during toxic *Pseudo-nitzschia* blooms, while the blue mussel showed no detectable toxin. In addition, a comparison of inter-animal variability (n = 60) in DA content revealed values ranging over an order of magnitude (*ca*. 0.5 to 5 micrograms DA/g tissue) and no consistent trend with size class, based on either animal weight or length. These data on the toxicity of individual animals will be useful in designing an appropriate sampling strategy for monitoring DA and, importantly, indicate that mole crabs do not appear to progressively bioaccumulate DA with age.

** NEW!!! ***Emerita analoga* (STIMPSON)-POSSIBLE NEW INDICATOR SPECIES FOR THE PHYCOTOXIN DOMOIC ACID IN CALIFORNIA COASTAL WATERS** (Toxicon 40:1259-1265) **…**M.E. Ferdin, R.G. Kvitek, C.K. Bretz, Christine Powell, Greg Doucette, K.A. Lefebvre, S. Coale and M.W. Silver.

ABSTRACT

Blooms of domoic acid (DA) synthesizing diatoms (*Pseudo-nitzschia* spp.) have been associated with the death and injury of hundreds of marine shorebirds and mammals, exposed humans to potentially serious health risks, and threatened to significantly impact coastal fisheries and economies dependent on marine resources. While indicator organisms are widely utilized to monitor for marine biotoxins like paralytic shellfish poisoning (PSP) toxins, a reliable intertidal indicator species to monitor DA remains to be identified. Here we evaluate and confirm the utility of the common sand crab (*Emerita analoga*) as an indicator for DA in comparison with sea mussels (*Mytilus californianus*). Mussels and sand crabs, collected from natural populations in Santa Cruz, California (Apr. 1999 - Feb. 2000), were tested for DA using the HPLC-UV method. Toxin loads in sand crabs ranged from below detectable limits to 10.4 µg DA g^{-1} and coincided with the abundance of DA producing *Pseudo-nitzschia* species nearshore. Toxin levels in mussels collected during the study period were below HPLC-UV detectable limits. The

rise and fall of DA in sand crabs in synchrony with *Pseudo-nitzschia* abundance, combined with this common intertidal species' accessibility and ease of DA extraction, recommend sand crabs as a reliable, cost-effective monitoring tool for DA in the coastal environment.

** NEW!!! **IMPACTS OF ALGAL TOXINS ON MARINE MAMMALS.** (Book chapter in Toxicology of Marine Mammals, Vos. J., Bossart, G.D., Fournier, M., O'shea, T., Eds. Taylor and Francis, New York, N.Y., p. 247-270)
…Fran VanDolah, Greg Doucette, F. Gulland, G. Bossart and T. Rowles.

ABSTRACT

Not available in electronic form at this time (see Fran)

** **MORPHOLOGY AND TOXICOLOGY OF *PROROCENTRUM ARABIANUM* SP. NOV., (DINOPHYCEAE) A TOXIC PLANKTONIC DINOFLAGELLATE FROM THE GULF OF OMAN, ARABIAN SEA** (Harmful Algae 1(4):393-400) … Steve L. Morton et al. (Elizabeth A. Fairey and Peter D. R. Moeller)

ABSTRACT

A new species of planktonic dinoflagellate, Prorocentrum arabianum isolated from the Gulf of Oman, is described using both scanning electron microscopy (SEM) and light microscopy. This clonal isolate has the following morphological characteristics: (1) cell shape is asymmetric; (2) thecal surface is rugose, covered with small poroids; (3) periflagellar area is unornamented, and (4) intercalary band is horizontally striated. Analysis of P. arabianum confirms the production of one cytotoxic compound and one ichthyotoxic compound. P. arabianum is the second known toxic planktonic Prorocentroid dinoflagellate.

** **REVIEW AND ASSESSMENT OF *IN VITRO* DETECTION METHODS FOR ALGAL TOXINS** (JAOAC International 84(5):1617-1626)… Fran VanDolah and John Ramsdell

ABSTRACT

Not available at this time (see Fran)

** **MODIFICATION OF THE CELL BASED ASSAY FOR BREVETOXINS USING HUMAN CARDIAC VOLTAGE DEPENDENT SODIUM CHANNELS EXPRESSED IN HEK-293 CELLS** (Biosensors & Bioelectronics 16:579-586)…Elizabeth Fairey et al.

ABSTRACT

Assays using living cells provide an effective means to generate activity measurements of toxins, especially in situations where the toxins are part of a complex mixture or in an unfamiliar form due to natural or synthetic derivatives or bioactive metabolites. An important step in the refinement of cell based assays is to simplify the cellular reactions to generate the functional response of interest. Advances in engineering functional responses in cells provide a means to direct the response of given toxins. In this report, we describe the homogeneous high level expression of the initial target for brevetoxin, the voltage dependent sodium channel in human embryonic kidney cells (HEK-293). HEK cells stably transfected with a 6.208 kb cDNA of human heart voltage-dependent Na+ channel (hH1a) were examined as an alternative to mouse neuroblastoma cells (N2A). The HEK-hH1a cells showed a reduced dependence on cofactors, increased sensitivity to brevetoxin and a useful means to assure absolute selectivity to the sodium channel. We next assessed the assay in a reporter gene format. Expression of a panel of minimal response elements as well as the c-fos promoter failed to provide a response to brevetoxin, indicating that the HEK cells lack a necessary intermediate signaling component. The expression of voltage dependent sodium channels in HEK cells is anticipated to provide enhanced performance for cell-based detection of toxins for drug and natural product discovery, biomonitoring and environmental monitoring.

**** CURRENT PROGRESS IN ISOLATION AND CHARACTERIZATION OF TOXINS ISOLATED FROM *Pfiesteria piscicida*** (Environmental Health Perspectives 109, suppl. 5:739-744)… Peter D. R. Moeller et al.

ABSTRACT

The isolation and purification of a toxic substances derived from *Pfiesteria piscicida* extracts are described. Four distinct bioassay systems were utilized to monitor bio-activity of the *Pfiesteria piscicida* extracts . These included a high throughput cell cytotoxicity assay and a reporter gene assay as well as assays using brine shrimp and fish. Using these bioassays to guide fractionation, we have isolated two distinct active fractions from *Pfiesteria* culture medium and cell mass extracts based on their solubility characteristics. We have identified and characterized a bio-active lipophilic substance from *Pfiesteria* derived extracts, as di-(2-ethylhexyl) phthalate (DEHP), a commonly used plasticizer. The source of this typically man-made substance has been identified as originating from Instant Ocean, (Instant Ocean, Aquarium Systems, Mentor OH) a commercially available seawater salt mixture used to make up our mass culture growth medium. We have developed chromatographic methodology to isolate a bio-active polar metabolite from the extracts and presently report the characterization of the activity of this substance. The molecular structural analysis of the polar active component(s) using mass spectrometry and nuclear magnetic resonance spectroscopy is currently underway.

**** CLASSIFICATION, NOMENCLATURE, AND IDENTIFICATION OF *Pfiesteria* AND *Pfiesteria*-LIKE SPECIES** (Environmental Health Perspectives 109, suppl 5:661-665)… Steidenger et al. (Steve Morton)

ABSTRACT

-See Steve Morton.

**** HEALTH AND ECOLOGICAL IMPACTS OF HARMFUL ALGAL BLOOMS: RISK ASSESSMENT NEEDS** (Human and Ecological Risk Assessment 7: 1329-1345) …Frances M Van Dolah et al.

ABSTRACT

The symposium session, *Indicators for Effects and Predictions of Harmful Algal Blooms*, explored the current state of indicators used to assess the human health and ecological risks caused by harmful algal blooms, and highlighted future needs and impediments that must be overcome in order to provide a complete risk assessment of their impacts. Six recognized human poisoning syndromes resulting from algal toxins (paralytic, neurotoxic, amnesic, diarrhetic shellfish poisonings, ciguatera fish poisoning, and putative estuary associated syndrome) impact human health through consumption of contaminated seafood, direct contact with bloom water, or inhalation of aerosolized toxin. Thorough health risk assessment for the variety of algal toxins is hampered to varying degrees because either the toxin has not been identified or indicators for exposure and effects remain poorly defined. Predicting the occurrence and determining the impacts of harmful algal blooms in coastal ecosystems are the two major ecological risk assessment needs. In the former case, the *hazard* is the suite of conditions that trigger bloom initiation, magnify bloom intensity or support bloom longevity, whereas in the latter case, the *hazard* is the algal toxin. In both cases, indicators (of triggering mechanisms, exposure, and effects) are better defined for some HAB species and toxins than others, but are by no means complete.

**** CELL CYCLE REGULATION IN A DINOFLAGELLATE, *Amphidinium operculatum*: IDENTIFICATION OF THE DIEL ENTRAINING CUE AND A POSSIBLE ROLE FOR CYCLIC AMP** (Journal of Experimental Marine Biology and Ecology 262:177-197) … Tod Leighfield and Frances M. Van Dolah

ABSTRACT

This research describes the diel phasing of the cell cycle in the dinoflagellate, *Amphidinium operculatum* Clapar☞de & Lachmann, and investigates the mechanisms that serve to link the cell cycle to the diel cycle. Unlike many dinoflagellates, *A. operculatum* has a high division rate of approximately 1 division□day $^{-1}$, that yields a nearly synchronous population, making it useful for population studies. When grown on a 16:8 h light:dark cycle, S-phase begins 10 hours and mitosis 14-16 h after the onset of light, as determined by flow cytometry. Alterations in the timing of the dark/light and light/dark transitions showed that the cell cycle is entrained by the dark/light transition, with the light/dark cue being uninvolved. Cells in logarithmic phase growth also undergo diel changes in cell size, reaching a maximum size late in the light phase, concurrent with mitosis. Stationary phase cells or cells blocked in G1 of the cell cycle with a cell cycle inhibitor, olomoucine, showed no size changes or reduced size changes over the diel cycle, suggesting a coupling of cell size to the cell division cycle. In *Euglena*, cAMP dependent signaling appears to mediate diel phasing of the cell cycle. Therefore, the role of cAMP in cell cycle control in *A. operculatum* was investigated. Measurement of intracellular cAMP by radioimmunoassay revealed that cAMP concentrations varied on a diel basis, but increases observed appeared to correlate with cell size increases, and did not correlate with light cues at the dark/light or light/dark transition. However, when cells were treated with the cAMP phosphodiesterase inhibitor, IBMX, cell cycle progression was inhibited at both the G1/S and the G2/M phase transitions. This is in agreement with the role of cAMP in the cell cycle control in higher eukaryotes and is the first report of the involvement of cAMP dependent signaling in the dinoflagellate cell cycle.